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ORIGINAL ARTICLE

# Evaluation of 10 years of parainfluenza virus, human metapneumovirus, and respiratory syncytial virus infections in lung transplant recipients

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Respiratory tract infection with pneumoviruses (PVs) and paramyxoviruses (PMVs) are increasingly associated with chronic lung allograft dysfunction (CLAD) in lung transplant recipients (LTRs). Ribavirin may be a treatment option but its effectiveness is unclear, especially with respect to infection severity. We retrospectively analyzed 10 years of PV/PMV infections in LTRs. The main end points were forced expiratory volume in 1 second ($FEV_1$) at 3 and 6 months postinfection, expressed as a percentage of pre-infection $FEV_1$ and incidence of new or progressed CLAD 6 months postinfection. A total of 139 infections were included: 88 severe infections (63%) (defined as >10% $FEV_1$ loss at infection) and 51 mild infections (37%) (≤10% $FEV_1$ loss). Overall postinfection CLAD incidence was 20%. Associations were estimated on postinfection $FEV_1$ for ribavirin vs no ribavirin (+13.2% [95% CI: 7.79; 18.67]) and severe vs mild infection (−11.1% [95% CI: −14.76; −7.37]). Factors associated with CLAD incidence at 6 months were ribavirin treatment (odds ratio (OR [95% CI]) 0.24 [0.10; 0.59]), severe infection (OR [95% CI] 4.63 [1.66; 12.88]), and mycophenolate mofetil use (OR [95% CI] 0.38 [0.14; 0.97]). These data provide valuable information about the outcomes of lung transplant recipients with these infections and suggests possible associations of ribavirin use and infection severity with long-term outcomes. Well-designed prospective trials are needed to confirm these findings.

KEYWORDS

antibiotic: antiviral, clinical research/practice, infection and infectious agents – viral, infectious disease, lung (allograft) function/dysfunction, lung transplantation/pulmonology

**Abbreviations:** 95% CI, 95% confidence interval; aOR, adjusted odds ratio; BOS, bronchiolitis obliterans syndrome; CLAD, chronic lung allograft dysfunction; eGFR, estimated GFR; $FEV_1$, forced expiratory volume in 1 second; hMPV, human metapneumovirus; RSV, respiratory syncytial virus; IQR, interquartile range; LDT, laboratory developed test; LTR, lung transplant recipient; PCR, polymerase chain reaction; PIV, parainfluenza virus; PMV, paramyxovirus; PV, pneumovirus; RSV, respiratory syncytial virus; uOR, unadjusted odds ratio; PIV, parainfluenza virus.

## 1 | INTRODUCTION

Respiratory syncytial virus (RSV) and human metapneumovirus (hMPV), members of the family *Pneumoviridae* (PV), and parainfluenza virus (PIV) types 1-4, members of the family *Paramyxoviridae* (PMV) are recognized increasingly as causes of serious morbidity and mortality.[1] This morbidity is especially pronounced in lung transplant recipients (LTRs), where an acute mortality due to these infections has been reported in up to 6%-20% of cases for individual viruses.[2-5] In addition, PV/PMV infections are associated increasingly with the development of chronic lung allograft dysfunction (CLAD).[3,6-10] CLAD is the primary factor limiting long-term survival after lung transplantation. It has a poor prognosis and is difficult to treat, which makes prevention paramount.[11,12] Unfortunately, CLAD incidence ranging from 25%-67% have been described after untreated PV/PMV infections in LTR.[5,6,8,9,13]

Although, these virus infections do not seem to increase the risk of acute rejection,[14,15] the severity of infection may be related to allograft dysfunction.[7,16]

Thus far the treatment options for these infections are limited, with some transplant centers currently using augmented steroids in combination with ribavirin.[17] Ribavirin is a nucleoside analogue antiviral that is currently only registered as inhalation therapy for severe RSV infection and is used off label (increasingly in oral form) based on its in vitro activity against hMPV and PIV 1-4.[18-20] Thus far, a limited number of small cohort studies have reported successful outcomes with ribavirin treatment for PV/PMV infections in LTR.[2,21-24] However, the effectiveness remains a matter of debate, as data from randomized studies are lacking. In this retrospective cohort study we aim to evaluate the course of lung function and incidence of new-onset or progression of CLAD in LTR with a PV/PMV infection, with or without ribavirin treatment.

## 2 | MATERIALS AND METHODS

### 2.1 | Patients

This is a retrospective cohort study. All adult patients with a single-, double-, or heart-lung transplantation who were at least 6 months posttransplant and were diagnosed with an RSV, hMPV, or PIV1-4 infection between 2008 and 2018 at the University Medical Centre Groningen were eligible for inclusion. If a patient had had multiple PV/PMV infections over the years, only the first 2 cases were included (which had to be at least 1 year apart, else only the first case was included). As part of regular care, chest x-ray and bacterial and fungal sputum cultures were performed in all patients to exclude other causes of lung function decline. Patients with factors that could interfere with reliable spirometry (eg, airway stenosis, chest wall pathology, pain), or who had a concomitant fungal infection, or had an episode of acute rejection within 3 months prior to PV/PMV infection were excluded.

The primary end point was the change in forced expiratory volume in 1 second ($FEV_1$) at 6months postinfection, as a percentage of preinfection $FEV_1$. Secondary end points were $FEV_1$ at 3 months postinfection (as a percentage of preinfection $FEV_1$), new-onset or progression of CLAD at 6 months postinfection, and change in hemoglobin level as a side-effect of ribavirin therapy. All patients had provided written informed consent for transplant-related research and the study was approved by the local ethics committee (METc 2015.452). The manuscript was prepared in accordance with the Strenghtening Reporting of Observational studies in Epidemiology statement.[25]

### 2.2 | Pulmonary function

Spirometry was performed according to American Thoracic Society/European Respiratory Society guidelines.[26] $FEV_1$ values at 4 time points were evaluated: preinfection, at infection, 3 months postinfection, and 6 months postinfection. Postinfection $FEV_1$ values were expressed as a percentage of the preinfection baseline value to determine recovery. Preinfection $FEV_1$ is defined as the average of all the $FEV_1$ values 4 months up to 2 weeks before infection. $FEV_1$ at infection is defined as the lowest $FEV_1$ during infection. Mild infections were defined as a ≤10% loss in this $FEV_1$ at infection compared to the average of 4 months preinfection, whereas a >10% loss in $FEV_1$ at infection compared to average of the 4 months preinfection was classified as severe infection.[5,8]

CLAD staging was characterized by a persistent (>3 months) $FEV_1$ decline of ≤80% of posttransplant *baseline*, with or without a change in forced vital capacity, according to the most recent International Society of Heart and Lung Transplantation criteria.[27] New-onset CLAD was classified as a new diagnosis of CLAD in a CLAD-naïve patient (eg, CLAD stage 0 to CLAD stage 1 or higher); progressed CLAD was defined as progression to a higher CLAD stage in a patient with preexisting CLAD.

### 2.3 | Virologic diagnostics

PV/PMV infection was defined as a positive polymerase chain reaction (PCR) test of a respiratory sample for RSV, hMPV, or PIV 1-4. Viral RNA detection was performed in nasopharyngeal swabs, nasal washes, sputum, or bronchoalveolar lavage specimens. RNA was extracted using the NucliSense EasyMag (bioMérieux). From 2008 until 2014, all respiratory samples were tested by a laboratory-developed real-time PCR-test (LDT), using 1xTaqMan Fast Virus 1-Step Master Mix (Applied Biosystems), as described previously.[28] From then onward the FilmArray respiratory panel (BioFire Diagnostics) was implemented in the laboratory alongside the LDT and used for priority testing of respiratory viruses, including RSV, hMPV, and PIV 1-4. Results were available within 24 hours after sample collection for the LDT and within 3 hours for the FilmArray panel.

### 2.4 | Management of PV/PMV infections

Treatment in case of PV/PMV infection consisted of oral prednisolone in combination with ribavirin or oral prednisolone only, and

was up to the discretion of the attending physician. Prednisolone was increased to 0.5 mg/kg/day in all patients for 7-10 days.[5,21,29] Suspected bacterial co-infection (indicated by purulent sputum production and/or infiltrate on chest x-ray without positive sputum culture) was treated up to the discretion of the attending physician. The immunosuppressive regimen was not changed based on the PV/PMV infection. Until 2012 aerosolized ribavirin was used, which was thereafter replaced by an oral ribavirin regimen (200 mg, b.i.d., if <70 kg, 400 mg, b.i.d., if >70 kg, both 10-14 days). In 2015, a full oral treatment protocol adapted from Burrows et al[22] was introduced, consisting of a high loading dose of ribavirin of 11 mg/kg t.i.d., in the first 24 hours, followed by a maintenance dose of 10 mg/kg, b.i.d., for 10-14 days. Maintenance dosing was halved in patients with an estimated GFR (eGFR) <50 mL/min. Anemia was classified as a hemoglobin level <13.0 g/dL for men or <12.0 g/dL for women.[30]

### 2.5 | Statistical analysis

Medians were compared using the nonparametric Mann-Whitney *U* test or the Kruskal-Wallis *H* test, proportions using the Fisher exact test or the chi-square test. $FEV_1$ 3- and 6-months postinfection was simultaneously analyzed using a hierarchical linear mixed-effects model. Time since transplantation, PV/PMV species (RSV, hMPV, or PIV), viral co-infection or not, bacterial co-infection or not, presence of CLAD pre-infection, severe or mild infection, follow-up time point (3 vs 6 months), tacrolimus use, mycophenolate mofetil use, and ribavirin treatment were selected as independent variables. Severe vs mild infection was defined by the acute $FEV_1$ loss during infection compared to the average of a period of 4 months pre-infection (see section 2.2.). By considering this variable in the model, a possible association of severe infection characterized by marked acute $FEV_1$loss during infection with long-term $FEV_1$ decline could be analyzed. The selection of variables was based on previously published risk factors, and a directed acyclic graph was constructed for assessment of potential confounding (Figure S1).[12,31] First, the most appropriate covariance structure was selected using restricted maximum likelihood and Akaike's information criterion. This was followed by variable selection using backward elimination, and lastly the final model was analyzed with restricted maximum likelihood to provide proper estimates of the associations of the variables with $FEV_1$ and their *P*-value. CLAD incidences were analyzed using multiple logistic regression with backward selection using the same independent variables. Variables are reported as unadjusted odds ratios (uORs) and adjusted odds ratios (aORs). Further elaboration of these methods is available in the Supplementary Material (Table S1-S4).

## 3 | RESULTS

### 3.1 | Patients and treatment

From December 2008 until March 2018, 115 individual patients tested positive in 159 events for a respiratory PV/PMV infection (24 patients with two cases were included). Twenty cases were excluded from analysis due to infection within 6 months posttransplant (n = 11), co-infected with invasive aspergillosis (n = 4), episode of acute rejection preceding infection (n = 4), or missing data (n = 1). Patient demographics and detected PV/PMV are shown in Table 1.

Most cases of PV/PMV infection occurred from December through March, with a lower incidence during the summer, when PIV was predominant (Figure 1). Initial infection was most often diagnosed in a nasopharyngeal swab sample (73%, mediancycle threshold value (Ct-value) 25; interquartile range [IQR] 10.0), followed by nasal wash (14%, median Ct-value 23; IQR 7.3), throat swab (6%, median Ct-value 28; IQR 6.5), bronchoalveolar lavage (4% median Ct-value 23; IQR 5.8) and sputum samples (1%, median Ct-value 24.5). The FilmArray panel was used in 22 nasopharyngeal swab samples, resulting in 16% of the total cases. Repeat viral testing after initial diagnosis was performed in 66 patients (48%) ranging from 7 to 30 days. Data on development of Ct-values in this timeframe are shown in Figure S1. Symptoms, $FEV_1$ loss at presentation and new radiologic abnormalities did not differ between the PV/PMV (Table 2). Supplemental oxygen therapy was required in 17% of cases (PIV 28%, RSV 13%, hMPV 9%). Chest x-ray abnormalities were mostly absent (72%) and nonspecific. Three patients died during follow-up (range 139-157 days postinfection), all unrelated to PV/PMV infection (1 renal failure/liver failure, 1 fasciitis necroticans, 1 metastasized squamous cell carcinoma). Ribavirin was primarily taken orally, comprising 85% of all ribavirin-treated cases. Median duration of treatment was 10 (IQR 4) days and 9 (IQR 3) days for oral or aerosolized ribavirin, respectively.

### 3.2 | Lung function and CLAD

Median $FEV_1$ at the different time points for the subgroups is illustrated in Figure 2. $FEV_1$ dropped notably during infection, with a median acute loss compared to preinfection $FEV_1$ of 14% (IQR 22), 15% (IQR 14), and 16% (IQR 20) for RSV, hMPV, and PIV, respectively. In total, 23 patients (17%) did not return to >90% of their preinfection $FEV_1$ value at 6 months postinfection (7 RSV, 6 hMPV, 10 PIV). For the multivariate analysis of long-term $FEV_1$ at 3 and 6 months postinfection, the variable selection approach identified ribavirin treatment (independent of time point), time since transplantation, their interaction, and severe infection as significant factors associated with long-term $FEV_1$ (Table 3). The other independent variables did not reach statistical significance or improved model fit and were omitted during the backward selection process (Table S1).

In the final model there was an overall positive association of ribavirin treatment vs no ribavirin treatment on long-term $FEV_1$ at 3 and 6 months postinfection of (estimate [95% confidence interval CI] 13.23% [7.79; 18.67]) and a negative association for severe vs mild infection of (estimate [95% CI] −11.07 [−14.76; −7.37]) (Table 3). The interaction between time since transplantation and ribavirin treatment suggested a weaker positive association for ribavirin treatment when patients with longer period after transplantation.

**TABLE 1** Patient demographics

| | All patients | Severe infection | | | Mild infection | | |
|---|---|---|---|---|---|---|---|
| | Total | No ribavirin | Ribavirin | *P* | No ribavirin | Ribavirin | *P* |
| Patients | 139 | 33 | 55 | | 35 | 16 | |
| Age at infection, y (IQR) | 54.1 (20) | 55.1 (13) | 54.8 (13) | .64 | 48.0 (23) | 54.9 (24) | .47 |
| Gender, female (%) | 72 (52) | 19 (56) | 26 (47) | .39 | 19 | 8 (50) | >.99 |
| Time since transplantation, y (IQR) | 3.9 (5) | 4.1 (5) | 3.9 (3) | .41 | 3.1 (6) | 4.0 (9) | .97 |
| Transplantation type: double, n (%) | 111 (80) | 29 (88) | 40 (73) | .12 | 28 (80) | 14 (88) | .70 |
| Single, n (%) | 26 (19) | 4 (12) | 1 3 (24) | .27 | 7 (20) | 2 (13) | .70 |
| Heart-lung, n (%) | 2 (1) | 0 (0) | 2 (4) | .53 | 0 (0) | 0 (0) | — |
| Underlying disease, n (%) | | | | | | | |
| COPD | 54 (39) | 15 (45) | 23 (42) | .83 | 11 (31) | 5 (31) | >.99 |
| Cystic fibrosis | 32 (23) | 5 (15) | 11 (20) | .78 | 11 (31) | 5 (31) | >.99 |
| Pulmonary hypertension | 11 (8) | 3 (9) | 3 (5) | .67 | 5 (14) | 0 (0) | .17 |
| Fibrosis | 20 (14) | 5 (15) | 9 (16) | >.99 | 3 (9) | 3 (19) | .36 |
| Alpha-1[a] | 10 (7) | 2 (6) | 5 (9) | .71 | 2 (6) | 1 (6) | >.99 |
| Other[b] | 12 (9) | 3 (9) | 4 (7) | >.99 | 3 (9) | 2 (13) | .64 |
| Virus, n (%) | | | | | | | |
| RSV | 40 (29) | 9 (27) | 15 (27) | >.99 | 9 (26) | 7 (44) | .33 |
| hMPV | 46 (33) | 12 (36) | 18 (33) | .82 | 11 (31) | 5 (31) | >.99 |
| Parainfluenza | 53 (38) | 12 (36) | 22 (40) | .82 | 15 (43) | 4 (25) | .35 |
| Parainfluenza type 1 | 14 (10) | 5 (15) | 3 (5) | .25 | 5 (14) | 1 (6) | .65 |
| Parainfluenza type 2 | 4 (3) | 1 (3) | 1 (2) | >.99 | 2 (6) | 0 (0) | >.99 |
| Parainfluenza type 3 | 26 (19) | 6 (18) | 13 (24) | .60 | 5 (14) | 2 (13) | >.99 |
| Parainfluenza type 4 | 9 (7) | 0 (0) | 5 (9) | .15 | 3 (9) | 1 (6) | >.99 |
| Co-infection, rhinovirus | 5 (4) | 0 (0) | 3 (5) | .29 | 1 (3) | 1 (6) | .53 |
| Coronavirus | 6 (4) | 1 (3) | 4 (7) | .65 | 0 (0) | 1 (6) | .31 |
| Bacterial | 6 (4) | 1 (3) | 2 (4) | >.99 | 2 (6) | 1 (6) | >.99 |
| Immunosuppressive medication, n (%) | | | | | | | |
| Cyclosporin | 6 (4) | 3 (9) | 2 (4) | .36 | 1 (3) | 0 (0) | >.99 |
| Tacrolimus | 124 (89) | 26 (79) | 52 (95) | .04 | 32 (91) | 14 (88) | .64 |
| Mycophenolic acid | 109 (78) | 26 (79) | 44 (80) | >.99 | 27 (77) | 12 (75) | >.99 |
| Azathioprine | 15 (11) | 4 (12) | 5 (9) | .72 | 5 (14) | 1 (6) | .65 |
| mTOR inhibitor | 15 (11) | 4 (12) | 3 (5) | .42 | 5 (14) | 3 (19) | .69 |
| Pre-infection CLAD, n (%) | 41 (29) | 13 (39) | 17 (31) | .49 | 7 (20) | 4 (25) | .72 |
| FEV1 at presentation[c] (IQR) | 84.7 (18) | 81.0 (11) | 78.0 (15) | .15 | 96.8 (5) | 94.7 (5) | .32 |
| Symptom onset to diagnosis, d | 7 (5) | 7 (9) | 6 (3) | .15 | 7 (5) | 4 (10) | .13 |

*Note:* Continuous data are displayed as medians with interquartile range (IQR).

Abbreviations: hMPV, human metapneumovirus; mTOR, mammalian target of rapamycin; RSV, respiratory syncytial virus.

[a]Alfa-1 antitrypsin deficiency.

[b]Other underlying disease (histiocytosis, sarcoidosis, Stevens-Johnson syndrome, graft versus host disease, bronchiectasis).

[c]During infection, as percentage of average FEV1 four months preinfection.

There was an overall high CLAD incidence with 26 of 127 patients (20%) having developed new or progressed CLAD at 6 months post-infection (13 new CLAD, 13 progressed CLAD). CLAD incidence was higher in patients with a severe infection compared to mild infection (21/78 [27%] vs 5/49 [14%], $P = .03$). There were no patients who developed restrictive allograft syndrome (RAS, or rCLAD) during the follow-up period after infection. Univariate analysis showed a lower incidence of newly developed CLAD in patients treated with

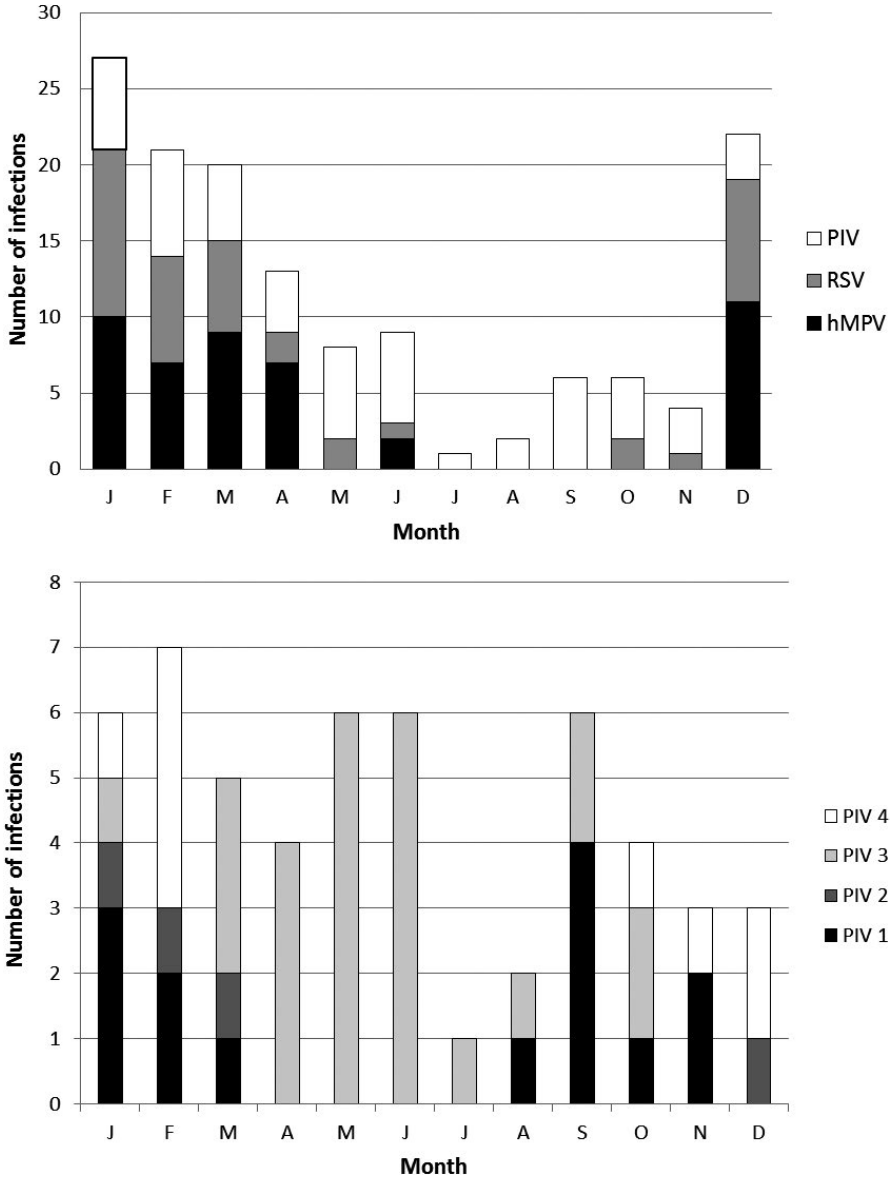


**FIGURE 1** Seasonal distribution of PV/PMV infections. hMPV, human metapneumo virus; PIV, parainfluenza virus; RSV, respiratory syncytial virus

ribavirin for severe infection, whereas this difference was not significant for progressed CLAD or patients with mild infection (Table 4). In case of severe infection PIV had the highest CLAD incidence with 10 of 29 patients (34%), followed by RSV (5/20, 25%) and hMPV (6/29, 21%); however, these differences were not statistically significant ($P$ = .47). CLAD incidences per individual virus and treatment group are available in Table S5.

The multiple logistic regression analysis of new or progressed CLAD at 6 months yielded findings similar to the long-term $FEV_1$ end point, with an association identified for severe infection and ribavirin treatment. There was a strong association between severe infection and development of new or progressed CLAD (uOR [95% CI] 2.89 [1.14; 7.29]; aOR [95% CI] 4.63 [1.66; 12.88]), whereas ribavirin treatment was associated with a lower incidence (uOR [95% CI] 0.37 [0.16; 0.85]; aOR [95% CI] 0.24 [0.10; 0.59]). In addition, the use of mycophenolate mofetil as part of standard immunosuppression was associated with a lower rate of new or progressed CLAD (uOR [95% CI] 0.46 [0.19; 1.12]; aOR [95% CI] 0.38 [0.14; 0.97]. We found no differences in outcome between the different ribavirin regimens in an exploratory analysis (Figure S3).

Hemoglobin levels during treatment were available in 64 patients treated with ribavirin (94%) and 28 patients in the non-ribavirin subgroup (39%) and are reported in Table 5. Five patients treated with oral ribavirin developed de novo or progressive anemia during infection (9%), 2 of whom required blood transfusion and therapy cessation.

## 4 | DISCUSSION

This study showed that PV/PMV infection was associated with a high CLAD incidence for all studied viruses, and the severity of infection was associated with recovery of lung function with an increased likelihood of new or progressed CLAD in case of severe infection compared to mild infection. It is encouraging that ribavirin treatment showed a positive association with a reduced CLAD incidence as well as $FEV_1$ recovery.

**TABLE 2** Symptoms and radiologic findings

| N, (%) | RSV (n = 40) | hMPV (n = 46) | PIV (n = 53) |
|---|---|---|---|
| Severe infection | 24 (60) | 30 (65) | 34 (64) |
| Hospitalized | 15 (38) | 17 (37) | 24 (45) |
| Median FEV1 at presentation[a] (IQR) | 85.6 (22) | 85.0 (14) | 84.0 (20) |
| Respiratory symptoms | 40 (100) | 46 (100) | 53 (100) |
| Cough | 33 (83) | 38 (83) | 48 (91) |
| Sputum | 19 (48) | 28 (61) | 34 (64) |
| Coryza | 19 (48) | 19 (41) | 19 (36) |
| Fever | 4 (10) | 12 (26) | 7 (13) |
| Dyspnea | 17 (43) | 12 (26) | 28 (53) |
| Sore throat | 7 (18) | 2 (4) | 3 (6) |
| Additional oxygen requirement | 5 (13) | 4 (9) | 15 (28) |
| Chest X-ray abnormalities | 10/36 (28) | 11/43 (26) | 13/50 (26) |
| Infiltrate | 3 (8) | 1 (2) | 3 (6) |
| Pleural effusion | 3 (8) | 4 (9) | 2 (4) |
| Consolidation | 2 (6) | 3 (7) | 4 (8) |
| Bronchial cuffing | 0 (0) | 1 (2) | 2 (4) |
| Increased interstitial markings | 2 (6) | 3 (7) | 2 (4) |
| Increased pulmonary vasculature | 1 (3) | 2 (5) | 0 (0) |

Abbreviations: hMPV, human metapneumo virus; PIV, parainfluenza virus; RSV, respiratory syncytial virus.

[a]As percentage of preinfection FEV1.

The yearly incidence of PV/PMV infections in our study was approximately 5%, which is comparable with earlier studies.[5,9] Because patients in our center are only tested on clinical indication instead of routine screening, the real incidence may be higher especially for mild or asymptomatic PV/PMV infection.[9,10,21] The impact of these asymptomatic or mild infections is unclear, however, which is underscored by the outcome in our mild infection cohort. Outcome in these patients was better than in patients with a severe infection, regardless of antiviral treatment. This is analogous to other reports of mild infection.[13,16,32] Therefore, not diagnosing subclinical infections may not be significantly detrimental for the allograft function of patients and therefore warrant abstinence of antiviral treatment.

A recent large study by Allyn et al[7] investigating viral respiratory infections in LTR emphasized this nuance in severity of infection by showing that viral pneumonia (indicated by chest x-ray infiltrate) increased the risk of allograft dysfunction or graft loss, while asymptomatic infection or symptomatic infection without radiographic infiltrates did not. Another recent study found that viral lower respiratory tract infection (defined based on lower tract symptoms or radiography) but not positivity for a virus in general, is a risk factor for CLAD development.[16] This emphasizes the need to focus on severity of infection rather than sample positivity as the sole factor. Most of our patients had no radiographic abnormalities at presentation despite a pronounced acute $FEV_1$ loss. An acute $FEV_1$ loss may be indicative of infection of the lower respiratory tract with activation of the immune response causing inflammation of the small airways yielding swelling and limiting airflow, thereby forming a risk factor for worse outcome.[33-36] Moreover, because PV/PMV infections often do not show radiographic abnormalities, the amount of acute $FEV_1$ loss may be a useful clinical marker for severe or mild infection and could guide treatment decisions.

The impact of PIV infections is described far less in LTR compared to hMPV and RSV. We found here that PV/PMV type was not a significant factor in our multivariate models and that severe infections were equally often caused by RSV, hMPV, and PIV. Furthermore, PIV was not only the most commonly detected agent, but severe infection, supplemental oxygen requirement, and CLAD incidence were all highest in cases with PIV. As such, infection with PIV should not be underestimated and be regarded of at least similar severity as hMPV and RSV.

Although ribavirin treatment has been reported for all PV/PMV in LTRs, with most literature concerning RSV, current evidence is mostly limited to small studies and univariate analyses, thereby limiting assessment of its efficacy.[2,5,9,13,22,37] To our knowledge, a study by Fuehner et al is currently the only available prospective study comparing ribavirin vs no ribavirin in LTRs with a PV/PMV infection.[5] They compared LTRs who received oral ribavirin (n = 38) for RSV, hMPV, or PIV infection to those who did not due to contraindications (n = 29) and found a lower incidence of new CLAD at 6 months in the ribavirin group (5% vs 24%, $P$ = .02, uOR [95% CI] 0.17 [0.03; 0.88]). In contrast, high CLAD incidences up to 20% have also been described in LTRs who received systemic ribavirin for RSV or hMPV.[4] Furthermore, although successful treatment of hMPV and PIV has been described,[5,6,21,24,38] no consensus exists whether to treat these infections with ribavirin.

Our study had several strengths to address these questions and assess the impact of PV/PMV infections and to make an estimation of associations of ribavirin treatment with outcome. The large sample size of the cohort and regularly scheduled follow-up at our center resulted in detailed clinical data that allowed a multivariate analysis, taking into account multiple infectious and non-infectious factors that could influence outcome, thereby minimizing confounding.[7,12,31] Using this strategy our study showed that ribavirin treatment, independent of the individual virus or other relevant factors, was associated with better long-term recovery of $FEV_1$ postinfection and lower CLAD incidence. The interaction between ribavirin treatment and time since transplantation was likely attributable to ribavirin treated patients with an infection more recently after transplantation and may still have a slightly improving pulmonary function after transplantation.

Regarding the individual viruses, CLAD incidence was lower in case of ribavirin treated PIV whilst a trend was observed in case of ribavirin treated RSV or hMPV compared to no ribavirin. The high

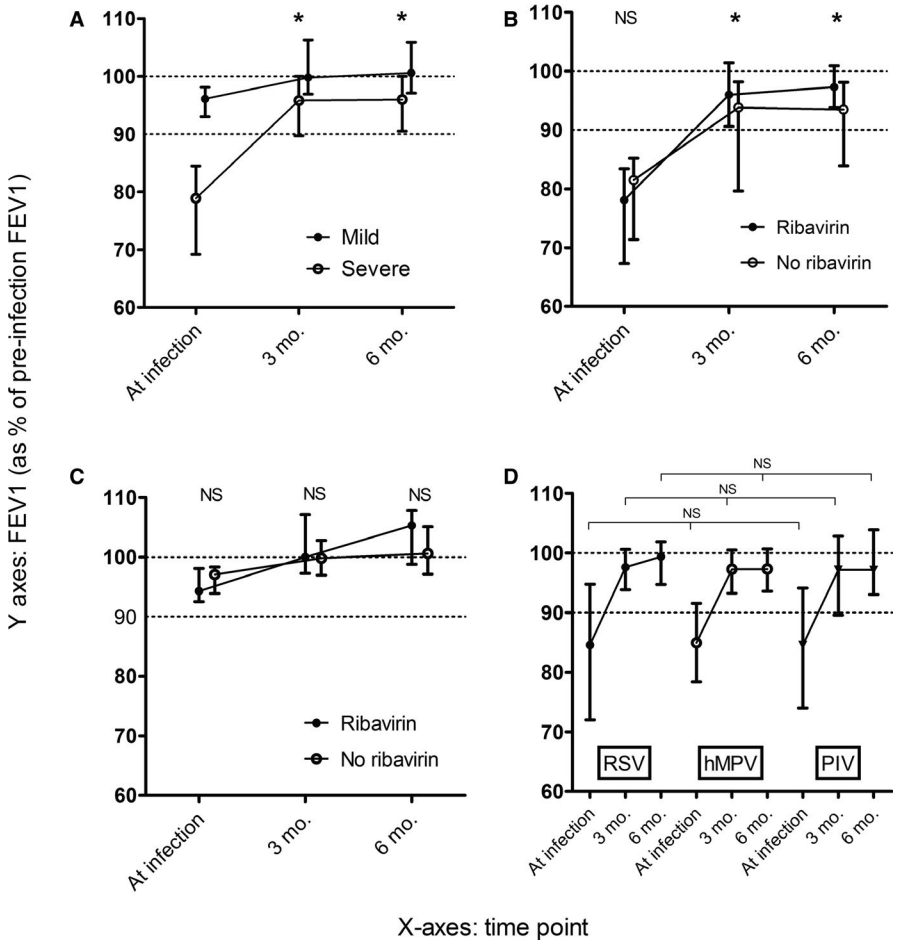


**FIGURE 2** FEV1 development over time per subgroup. FEV1 presented as percentage of pre-infection FEV1 for: (A) mild vs severe infection, (B) severe infection: ribavirin vs no ribavirin, (C) mild infection: ribavirin vs no ribavirin, (D) PV/PMV type. Data presented as medians + interquartile range. Asterisks indicate statistically significant differences from the Mann-Whitney U test, NS, not significant

**TABLE 3** Associations of variables on FEV1 at 3 and 6 months postinfection

| | Estimate [95% CI] | *P* value |
|---|---|---|
| Period (6 mo vs 3 mo) | 0.480 [−1.701; 0.741] | .440 |
| Severe infection (vs mild infection) | −11.07 [−14.76; −7.37] | <.001 |
| Ribavirin treatment (vs no ribavirin) | 13.23 [7.79; 18.67] | <.001 |
| Time after transplantation | | |
| No ribavirin | 0.427 [−0.117; 0.970] | .130 |
| Ribavirin | −0.709 [−1.322; −0.096] | .024 |

*Note:* Estimates are expressed as postinfection FEV1 as percentage of preinfection FEV1. Results of final hierarchical linear mixed model shown, full model available in Supplementary Material.

Abbreviation: CI, confidence interval.

**TABLE 4** Univariate analysis of CLAD incidences

| | Severe infection | | | Mild infection | | |
|---|---|---|---|---|---|---|
| n, (%) | No ribavirin | Ribavirin | *P* | No ribavirin | Ribavirin | *P* |
| New CLAD | 7/19 (35) | 3/37 (8) | .01 | 2/28 (7) | 1/12 (8) | >.99 |
| Progressed CLAD | 7/10 (70) | 4/12 (33) | .20 | 2/5 (40) | 0/4 (0) | .44 |
| Total CLAD | 14/29 (48) | 7/49 (14) | <.01 | 4/33 (12) | 1/16 (6) | .66 |

*Note: P* value from Fishers exact test.

Abbreviation: CLAD, chronic lung allograft dysfunction.

**TABLE 5** Hemoglobin levels

| | Hb pre-infection | Hb decrease during infection | *P* value |
|---|---|---|---|
| Oral ribavirin (n = 56) | 12.3 (1.45) | 1.1 (1.5) | <.01 |
| Aerosolized ribavirin (n = 8) | 13.2 (1.6) | 0.3 (0.8) | .36 |
| No ribavirin (n = 28) | 12.4 (1.45) | 0.5 (0.8) | .01 |

*Note:* Hb is reported as g/dL, data reported as: mean (standard deviation), *P* value is from the paired samples *t*-test.
Abbreviation: Hb, hemoglobin.

CLAD incidence of untreated severe PIV infection in our study of 66% is comparable with two small studies reporting incidences of 57% and 66% of untreated PIV cases.[8,9]

Given the possible detrimental effects of PV/PMV infection in LTR, and the in vitro susceptibility to ribavirin of PV/PMV, we believe ribavirin treatment for RSV, hMPV, and PIV may be considered an option until evidence from randomized controlled trials about its effectiveness or other treatment options are available.

Interestingly, we found a protective effect of mycophenolate mofetil as standard immunosuppression for the CLAD end point, but not for the $FEV_1$ end point. Mycophenolate mofetil is the prodrug for mycophenolic acid, which has shown intrinsic antiviral potential against, amongst others, hepatitis C and E viruses, and PIV-3, through a similar antiviral mechanism as ribavirin.[39-41]

Having ambiguous results for the two different end points and a broad confidence interval, we cannot be certain there was a true influence of mycophenolate mofetil on graft function. However, this finding certainly warrants further investigation into the antiviral properties of mycophenolic acid, its interplay with ribavirin and the balance with immunosuppression.

We do note certain limitations exist inherent to the study design such as a selection bias for patients who were more critically ill and the lack of randomization, thereby limiting causal inference. In addition, different ribavirin treatment regimens have been used in our study; however, several studies showed comparable results using different treatment regimens for RSV.[22,23,37,42,43] Finally, although we considered alternative factors explaining the outcomes in the ribavirin group, we did not identify associations for these factors. Nevertheless, we cannot exclude some degree of residual confounding even with careful covariate selection based on previous literature and use of multivariate techniques. It is unlikely, however, that this will be of such magnitude that it would invalidate the main associations.

Considering the increased recognition of the importance of these non-influenza viral infections and the current state of evidence, a multicenter randomized controlled trial would be the next step to evaluate the true value of ribavirin for PV/PMV infections in LTRs. This is also important in light of upcoming new therapeutic options which should be compared to currently available options.

In conclusion, our data provide valuable information about the outcomes of LTRs with PV/PMV infections and suggests possible associations for ribavirin use and infection severity with long-term outcomes. However, well-designed prospective trials are needed to confirm these findings.

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## DISCLOSURE

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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